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Effects of remote ischemic postconditioning treatment on ferroptosis and hexokinase II levels in a rat model of cerebral ischemia-reperfusion injury

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MAIN POINTS

- Following I/R, Increased levels of the ferroptosis marker ALOX12 and GPX4 suggest that ferroptotic cell death contributes to the injury.
- The neuroprotective mechanism of RePostC may be associated with the reduction of ALOX12 levels and the elevation of GPX4 levels.
- GPX4, ALOX12, LCN2, and HKII have been identified as novel biomarkers and therapeutic targets for cerebral I/R injury.
- RePostC's protective effect is linked to the activation of internal tolerance mechanisms and the strengthening of antioxidant defenses.

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ABSTRACT

Aim: Cerebral ischemia-reperfusion (I/R) injury is an unavoidable outcome of reperfusion therapy following a stroke, initiating intricate death mechanisms at both cellular and molecular levels. Comprehending the molecular pathways that result in neuronal death post-stroke is crucial for the development of effective neuroprotective strategies. Ferroptosis is a mechanism of cell death characterized by iron-dependent lipid peroxidation and is believed to contribute to the damage associated with cerebral I/R injury.

Materials and Methods: Thirty male Sprague-Dawley rats were randomly assigned to three experimental groups: Control, IR, and IR+REPOSTC (n=10). Cerebral ischemia was induced using the intraluminal filament technique for 60 minutes, and the animals were euthanized after a 24-hour reperfusion period. The femoral artery of the right hind limb of the rat underwent RePostC consisting of three cycles of 5 minutes of ischemia followed by 5 minutes of reperfusion. Triphenyl tetrazolium chloride (TTC) staining was used to measure infarct size.

Results: Compared to the control group, the I/R group showed a significant increase in glutathione peroxidase 4 (GPX4) levels, while RePostC treatment significantly decreased GPX4 levels. Compared to the control group, the I/R group showed a significant decrease in hexokinase II (HKII) levels. The RePostC group, however, exhibited a substantial increase in these levels compared with the I/R group. Levels of arachidonic acid 12-lipoxygenase (ALOX12) and lipocalin 2 (LCN2) were significantly increased in the I/R group compared with the control group. No statistically significant difference was observed between the RePostC treatment group and the I/R group.

Conclusion: Ferroptotic cell death plays a role in the damage that occurs after cerebral I/R. GPX4, ALOX12, LCN2, and HKII levels may serve as potential targets for the management of cerebral ischemia-reperfusion injury. The RePostC application may help protect against I/R injury by activating the body's own tolerance mechanisms.

Keywords: Cerebral I/R, Ferroptosis, GPX4, ALOX-12

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INTRODUCTION

Stroke is one of the leading causes of death and illness worldwide, and its incidence is rising [1]. Ischemic stroke is the most common type of stroke, accounting for approximately 87% of cases. It occurs when blood flow to the brain suddenly stops because thrombosis or embolism blocks the blood vessels that supply it. Statistics show that more than 14 million people are affected by this condition each year [2]. Thrombolysis with tissue plasminogen activator (tPA), which targets reperfusion, and mechanical thrombectomy are the two most common treatments for ischemic stroke. However, these methods don't work well enough to treat strokes [3]. Reperfusion can exacerbate injury in ischemic tissue, causing ischemia/reperfusion (I/R) injury [4]. I/R injury induces cell

death, thereby exacerbating damage to ischemic tissue. This is followed by the infiltration of neutrophils, the accumulation of reactive oxygen species (ROS), the disruption of cellular ion homeostasis, and the activation of inflammatory responses [5].

The mechanisms responsible for cerebral I/R injury are not fully elucidated; nevertheless, emerging evidence suggests that ferroptosis significantly contributes to this phenomenon [6]. Ferroptosis is a regulated mechanism of cell death characterized by iron-dependent lipid peroxidation and is genetically, morphologically, and biochemically distinct from other forms of cell death [7].

Extensive research in recent years has examined mechanisms regulating ferroptosis, including the roles of iron, lipids, and amino acids [8]. GPX4 plays a role in ferroptosis and in amino acid metabolism by reducing lipid hydroperoxides to lipid alcohols. This inhibits the formation of toxic lipid ROS that are dependent on iron (Fe^{2+}) [9]. In addition to blocking hydroperoxide-dependent processes, GPX4 inhibits the ALOX (arachidonic acid lipoxygenase) family. This family is thought to be involved in the iron metabolism pathway in ferroptosis [10,11].

ALOX12 (arachidonic acid 12-lipoxygenase), a member of the lipoxygenase family, is involved in pro-oxidant and pro-inflammatory processes [12]. Nonetheless, the function of ALOX in lipid peroxidation resulting from GPX4 deficiency is yet to be elucidated.

Lipocalin-2 (LCN2) is an acute-phase protein that binds siderophores released by microorganisms, thereby sequestering iron. Its levels increase during infections or after various traumas [13,14]. Research indicates that LCN2 diminishes ferroptotic cell death [15]. Patients who have experienced ischemic stroke exhibit increased plasma levels of LCN2 [16]. Nonetheless, the function of LCN2 in stroke and the impact of RePostC on LCN2 remain incompletely understood.

The enzyme that phosphorylates glucose to form glucose-6-phosphate [17] is hexokinase II (HK-II). This is the first step in glycolysis. HK-II attaches to the outer mitochondrial membrane via the Voltage-Dependent Anion Channel 1 (VDAC1). This causes HK-II to dissociate from the mitochondria, leading to cell death [18]. Research indicates that elevated HK-II expression confers neuroprotective effects in a model of cerebral ischemia, reduces oxidative stress, and preserves mitochondrial stability [19].

Remote ischemic postconditioning (RePostC) is a noninvasive technique designed to safeguard vital organs compromised by ischemia via interventions administered to remote body regions [20]. Its accessibility and noninvasive nature have demonstrated its therapeutic potential across numerous conditions, encompassing ischemic neurological disorders in the acute, subacute, and chronic phases, including acute ischemic stroke [21].

This study investigated the role of ferroptosis in cerebral I/R injury, highlighting interactions of GPX4, ALOX12, and LCN2 with mitochondrial HKII. We also sought to clarify the regulatory impacts of RePostC on GPX4, ALOX12, LCN2, ferroptosis, and HKII at the molecular level to inform treatment strategies for cerebral I/R injury. Our study is the first to show how RePostC affects the levels of ALOX12, LCN2, and HKII. The RePostC application inhibited ferroptotic cell death by elevating GPX4 and HKII levels and exhibited a substantial neuroprotective effect against cerebral I/R injury.

MATERIALS AND METHODS

Statement of ethics and animal care

The Fırat University Faculty of Medicine Animal Experimentation Ethics Committee (Date: 21.06.2023; Protocol No: 2023/12-07) established and approved the ethical rules governing all animal experiments. The sample size analysis, conducted prior to the study using the "Web-based sample size and power analysis software" [22], indicated that at least 18 rats (6 per group) should be included to evaluate the parameters (power = 80%, $\alpha = 0.05$, $\beta = 0.2$). To increase the power of the study and minimize mortality from surgical procedures, 30 male Sprague-Dawley rats aged 10 to 12 weeks were used. The rats lived in a room with a temperature range of 22 ± 1°C and a 12-hour light/dark cycle. Animals had ad libitum access to food and water. The study used the minimum number of animals required, and measures were implemented to mitigate pain and discomfort.

Groups and experimental design

The study involved weighing all rats and randomly assigning them to three groups of 10 animals each: the Control Group (C), the Ischemia-Reperfusion Group (IR), and the Ischemia-Reperfusion and Remote Ischemic Postconditioning Treatment Group (IR+RePostC). In the IR+RePostC group, three cycles of 5-minute ischemia followed by 5-minute reperfusion were applied to the femoral artery in the right hind leg at the onset of reperfusion.

Initiation of the experimental cerebral ischemia-reperfusion model

Control, IR, and IR+RePostC rats underwent occlusion of the right middle cerebral artery (MCA) via the Koizumi intraluminal filament technique [23], thereby initiating a 60-minute period of ischemia. The animals were given ketamine-xylazine [24] to induce anesthesia, and their body temperature was maintained between 36.5°C and 37°C throughout the protocol. The right common carotid artery (CCA), the carotid bifurcation, and the external and internal carotid arteries (ECA and ICA) were carefully exposed by cutting away the surrounding tissues. The ECA was tied off distally, the CCA was tied off proximally, and a temporary microvascular clamp was placed on the ICA. A 4/0 nylon filament with

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a silicone-coated tip was inserted into the ICA through a incision in the right CCA. After the clamp was removed, the filament was gently advanced until it met resistance (approximately 18–20 mm), indicating occlusion of the middle cerebral artery (MCA). The filament stayed in place for

It was applied for an hour to induce ischemia, and then it was taken out to allow reperfusion. The same surgical steps were performed on the control animals, but no filament was inserted. After the procedure, the incision was closed with sutures, and the animals were returned to their cages to recover.

Analysis of Western blots

We used Western blotting to assess HKII levels. Western blot analysis of tissue HKII levels was performed using the appropriate antibodies. Before use, primary antibodies were mixed with a buffer containing 0.05% Tween-20 and then diluted 1:1000. We incubated nitrocellulose membranes with primary antibodies at +4°C overnight. Subsequently, the nitrocellulose membranes were washed five times, each wash lasting five minutes, with a buffer. After washing, the nitrocellulose membranes were incubated at 37°C for 90 minutes with goat anti-rabbit immunoglobulin conjugated to peroxidase, diluted 1:1000 in the same buffer. Lastly, the washing process was performed five times, each lasting five minutes. We used beta-actin for normalization.

Analyzing ELISA

The ELISA method was used to measure the levels of GPX4, ALOX12, and LCN2. Blood samples from the experimental groups were allowed to coagulate at room temperature for 30 minutes and then centrifuged at 5000 rpm for 15 minutes to separate the serum. The collected serum samples were stored at -80°C until testing. Using ELISA kits from BTLab (Shanghai Korain Biotech Co., Ltd., China), we measured the serum levels of the proteins GPX4, ALOX12, and LCN2 according to the manufacturer's instructions.

The kits were purchased with the following catalog numbers: GPX4 (Cat. No. YLA1783RA), ALOX12 (Cat. No. E3428Ra), and LCN2 (Cat. No. E1434Ra).

Statistical analysis

The data were analyzed using the IBM SPSS Statistics version 21.0 (Armonk, NY: IBM Corp.) , and the results are presented as mean ± standard deviation. The Shapiro-Wilk test was used to assess normality, and one-way ANOVA followed by Tukey's post hoc test was used to assess differences between groups. The correlation analysis employed Pearson's correlation test and two-tailed significance tests, with a significance threshold set at $p<0.05$.

RESULTS

GPX4

The IR group (11.57 ± 0.67) had significantly higher GPX4 protein levels than the control group (7.35 ± 0.13) ($p = 0.0003$). The IR+RePostC group (7.85 ± 0.13) had significantly lower GPX4 levels compared with the IR group ($p = 0.006$), but there was no significant difference between the control group and the IR+RePostC group ($p = 0.30$) (Figure 1).

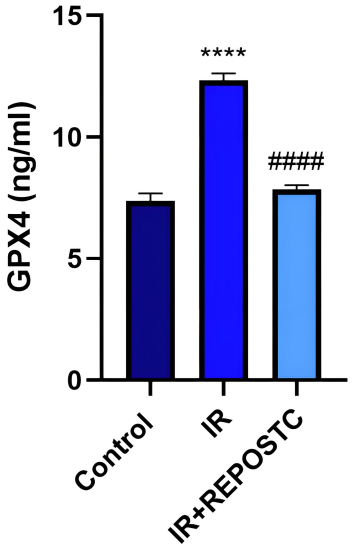

Figure 1. Impact of groups on GPX4 concentrations. ****p<0.0001 when compared to control, and ####p<0.0001 when compared to the IR group.

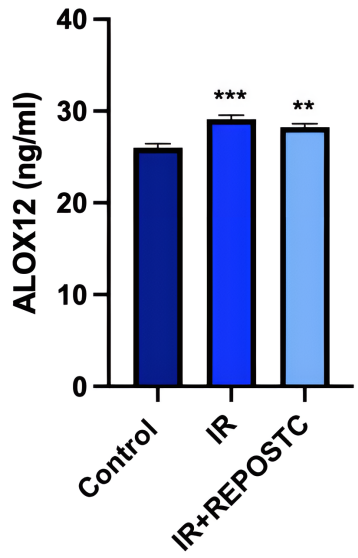

Figure 2. The impact of groups on ALOX12 levels. **p<0.01 in relation to control.

ALOX12

The levels of ALOX12 protein were statistically significantly higher in the IR (29.10 ± 0.45) and IR+RePostC (28.21 ± 0.37) groups than in the control group (26.01 ± 0.43) ($p = 0.0003$). No statistically significant changes were observed in the IR+RePostC group compared with the IR group ($p = 0.30$) (Figure 2).

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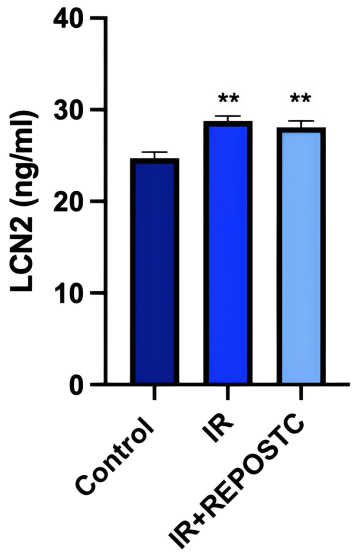

Figure 3. The influence of groups on LCN2 levels. **p<0.01 compared to control.

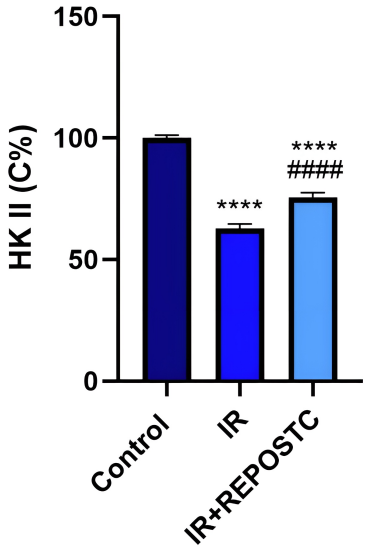

Figure 4. Impact of groups on HKII levels. ****p<0.0001 in comparison to control, ####p<0.0001 in comparison to the IR group.

LCN2

The IR (28.79 ± 0.52) and IR+RePostC (28.09 ± 0.68) groups had significantly higher levels of LCN2 protein than the control group (24.70 ± 0.67) (p = 0.0018). No statistically significant difference was observed between the IR+RePostC and IR groups (p = 0.72) (Figure 3).

HKII

The IR group (62.82 ± 1.92) had significantly lower levels of HKII protein than the control group (100.0 ± 1.13) (p<0.0001). In the IR+RePostC group (75.71 ± 1.79), HKII levels were statistically significantly elevated in comparison to the IR group (p<0.0001) (Figure 4).

DISCUSSION

This study investigated the role of ferroptosis in cellular death pathways related to cerebral I/R injury, the protective effects of remote postconditioning as a mechanical intervention, and its influence on the levels of GPX4, ALOX12, LCN2, and HKII. In the I/R group, HKII were significantly lower compared to the control group, while GPX4 levels were significantly higher. After RePostC administration, the increase in HKII levels and the decrease in GPX4 levels were significant compared to the I/R group. The I/R group exhibited substantially higher levels of ALOX12 and LCN2 than the control group. There was no statistically significant difference between the RePostC treatment group and the I/R group.

Ferroptosis is a regulated form of cell death characterized by iron-dependent lipid peroxidation [25]. Ferroptosis involves the iron-dependent production of lipid reactive oxygen species (L-ROS), the depletion of glutathione (GSH), the inactivation of glutathione peroxidase 4 (GPX4), the disturbance of cellular antioxidant equilibrium, and the consequent accumulation of lipid peroxides, leading to cell death [26].

GPX4, an important antioxidant enzyme, reduces lipid peroxides by converting membrane lipid hydroperoxides (L-OOH) to lipid alcohols (L-OH) with the help of GSH, thereby maintaining membrane stability [27]. GPX4 levels decrease during post-stroke ferroptosis, and upregulation of GPX4 may protect neuronal function from ferroptosis-induced damage [28]. However, Wenyuan et al. reported that GPX4 levels increased following I/R injury [29]. Similar to this finding, our study showed an increase in GPX4 levels after I/R injury; however, a significant decrease was observed in the RePostC-treated group compared to the I/R group. The increase in GPX4 levels may result from an acute adaptive stress response initiated by the cell to protect itself from ferroptosis caused by lipid peroxidation. In contrast, the decrease in GPX4 levels in RePostC-treated groups is thought to be a secondary effect of the treatment's successful reduction of early-stage ROS production and lipid peroxidation.

RePostC. This suggests that RePostC may provide protection against ferroptosis by modulating GPX4 levels.

Ferroptosis is a process of lipid peroxidation caused by iron accumulation via non-enzymatic Fenton reactions. ALOX enzymes and related enzymatic pathways are primarily responsible for this.

ALOX12 induces ferroptosis by initiating lipid peroxidation via the Fenton reaction when Fe^{2+} levels are elevated. In our investigation, serum ALOX12 levels increased following I/R injury, whereas RePostC administration did not produce a statistically significant change. The literature indicates that ALOX12 is activated locally, especially in neuronal and glial cells, suggesting that systemic levels may not accurately represent these intracellular alterations. Future investigations of tissue-specific ALOX12 expression levels will enhance understanding of the mechanisms underlying RePostC.

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Impaired expression of LCN2 may lead to neuronal degeneration and cognitive dysfunction. LCN2 can exacerbate neuronal injury and apoptosis through mechanisms that increase oxidative stress and induce mitochondrial dysfunction [30].

Research indicates that LCN2 increases intracellular Fe2+ levels in neurons, thereby augmenting oxidative damage and promoting ferroptosis [31]. Previous studies have demonstrated that LCN2 facilitates astrocyte activation [32] and exacerbates inflammatory damage in cerebral ischemia via pro-inflammatory cytokines [33]. In our study, serum LCN2 levels increased after IR injury; however, the treatment did not produce a statistically significant change in those levels. RePostC's protective mechanism may not directly affect LCN2 expression. Serum LCN2 may not completely represent tissue alterations; localized neuronal LCN2 variations may occur independently of serum concentrations.

In a model of ischemic stroke, the elimination of HK-II has been shown to exacerbate inflammatory responses and augment cerebral damage. The pro-inflammatory effects observed following deletion of HK-II are thought to be associated with impaired mitochondrial function and accumulation of reactive oxygen species (ROS) [17]. Li et al. established an HK-II-dependent connection between hyperglycolysis and neuroinflammation, proposing that selective inhibition of HK-II induction may represent an innovative therapeutic approach for ischemic stroke [34]. In our study, mitochondrial HK-II levels diminished following I/R injury. This finding corroborates that mitochondrial dysfunction and cell death mechanisms linked to I/R injury are activated, aligning with existing literature on HK-II's role in safeguarding mitochondrial membrane integrity and in energy metabolism. HK-II levels were markedly increased in the groups receiving RePostC compared with the I/R group. According to these results, PostC and RePostC applications may exert neuroprotective effects by increasing HK-II levels.

CONCLUSION

The rise in ferroptosis markers GPX4 and ALOX12 levels post-I/R, suggests that ferroptotic cell death contributes to the injury. Blocking ALOX12 and LCN2 and increasing GPX4 levels may help prevent ferroptosis. The levels of GPX4, ALOX12, LCN2, and HKII may represent viable targets for therapeutic intervention in cerebral I/R injury. The neuroprotective effect of RePostC may be attributed to elevated levels of GPX4 and HKII. The administration of RePostC may induce endogenous tolerance mechanisms that offer protection against I/R injury.

Ethics Committee Approval: Experimental Animal Ethics Committee of Fırat University, Faculty of Medicine (Date: 21.06.2023; Protocol No: 2023/12-07).

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

1. Iadecola C, Buckwalter MS, Anrather J. Immune responses to stroke: Mechanisms, modulation, and therapeutic potential. *J Clin Invest.* 2020;130(6):2777–2788. doi: 10.1172/jci135530.
2. Taoufik E, Probert L. Ischemic Neuronal Damage. *Curr Pharm Des.* 2008;14(33):3565–73. doi: 10.2174/138161208786848748.
3. Tao T, Liu M, Chen M, et al. Natural medicine in neuroprotection for ischemic stroke: Challenges and prospective. *Pharmacol Ther.* 2020;216:107695. doi: 10.1016/j.pharmthera.2020.107695.
4. Widimsky P, Snyder K, Sulzenko J, Hopkins LN, Stetkarova I. Acute ischaemic stroke: recent advances in reperfusion treatment. *Eur Heart J.* 2023;44(14):1205–15. doi: 10.1093/eurheartj/ehac684.
5. Minutoli L, Puzzolo D, Rinaldi M, et al. ROS-Mediated NLRP3 Inflammasome Activation in Brain, Heart, Kidney, and Testis Ischemia/Reperfusion Injury. *Oxid Med Cell Longev.* 2016;2183026:1-10. doi: 10.1155/2016/2183026.
6. He Y, Wang J, Ying C, et al. The interplay between ferroptosis and inflammation: therapeutic implications for cerebral ischemia-reperfusion. *Front Immunol.* 2024;15:1–19. doi: 10.3389/fimmu.2024.1482386.
7. Wang P, Cui Y, Ren Q, et al. Mitochondrial ferritin attenuates cerebral ischaemia/reperfusion injury by inhibiting ferroptosis. *Cell Death Diseases*. 2021;12(5). doi: 10.1038/s41419-021-03725-5.
8. Liu X, Xie C, Wang Y, et al. Ferritinophagy and Ferroptosis in Cerebral Ischemia Reperfusion Injury. *Neurochem Res.* 2024;49(8):1965–79. doi: 10.1007/s11064-024-04161-5.
9. Forcina GC, Dixon SJ. GPX4 at the Crossroads of Lipid Homeostasis and Ferroptosis. *Proteomics.* 2019;19(18):1–11. doi: 10.1002/pmic.201800311.
10. Xie Y, Kang R, Klionsky DJ, Tang D. GPX4 in cell death, autophagy, and disease. *Autophagy.* 2023;19(10):2621–38. doi: 10.1080/15548627.2023.2218764.

https://doi.org/10.5455/annalsmedres.2025.10.309

11. Schnurr K, Belkner J, Ursini F, Schewe T, Kühn H. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase controls the activity of the 15-lipoxygenase with complex substrates and preserves the specificity of the oxygenation products. *J Biol Chem.* 1996;271(9):4653–8. doi: 10.1074/jbc.271.9.4653.
12. Dong X, Jiang D, Chen S, et al. KNC nanozyme repairs hypoxia ischemia brain damage through ALOX12 mediated lipid peroxidation inhibition. *Bioact Materials.* 2025;54:531–48. doi: 10.1016/j.bioactmat.2025.08.032.
13. Luo C, Zhou S, Yin S, et al. Lipocalin-2 and Cerebral Stroke. *Front Mol Neurosci.* 2022;15:1–9. doi: 10.3389/fnmol.2022.850849.
14. Jha MK, Lee S, Park DH, et al. Diverse functional roles of lipocalin-2 in the central nervous system. *Neurosci Biobehav Rev.* 2015;49:135–56. doi: 10.1016/j.neubiorev.2014.12.006.
15. Meier JK, Schnetz M, Beck S, et al. Iron-Bound Lipocalin-2 Protects Renal Cell Carcinoma from Ferroptosis. *Metabolites.* 2021;11(5):329. doi: 10.3390/metabo11050329.
16. Wang G, Weng YC, Han X, Whaley JD, Mccrae KR, Chou WH. Lipocalin-2 released in response to cerebral ischaemia mediates reperfusion injury in mice. *J Cell Mol Med.* 2015;19(7):1637–45. doi: 10.1111/jcmm.12538.
17. Hu Y, Cao K, Wang F, et al. Dual roles of hexokinase 2 in shaping microglial function by gating glycolytic flux and mitochondrial activity. *Nat Metab.* 2022;4(12):1756–74. doi: 10.1038/s42255-022-00707-5.
18. Shoshan-Barmatz V, Golan M. Mitochondrial VDAC1: Function in Cell Life and Death and a Target for Cancer Therapy. *Curr Med Chem.* 2012;19(5):714–35. doi: 10.2174/092986712798992110.
19. Wu B, Luo H, Zhou X, et al. Succinate-induced neuronal mitochondrial fission and hexokinase II malfunction in ischemic stroke: Therapeutical effects of kaempferol. *Biochim Biophys Acta Mol Basis Dis.* 2017;1863(9):2307–18. doi: 10.1016/j.bbadis.2017.06.011.
20. Hausenloy DJ, Yellon DM. The therapeutic potential of ischemic conditioning: An update. *Nat Rev Cardiol.* 2011;8(11):619–29. doi: 10.1038/nrcardio.2011.85.
21. Hess DC, Blauenfeldt RA, Andersen G, et al. Remote ischaemic conditioning-a new paradigm of self-protection in the brain. *Nat Rev Neurology.* 2015;11(12):698–710. doi:10.1038/nrneurol.2015.223.
22. Arslan AK, Yaşar Ş, Çolak C, Yasar S. WSSPAS: An Interactive Web Application for Sample Size and Power Analysis with R Using Shiny. *Biostat.* 2018;10(3):224-46. doi: 10.5336/biostatic.2018-62787.
23. Takano K, Tatlisumak T, Bergmann AG, Gibson DG, Fisher M. Reproducibility and reliability of middle cerebral artery occlusion using a silicone-coated suture (Koizumi) in rats. *J Neurol Sci.* 1997;153(1):8–11. doi: 10.1016/s0022-510x(97)00184-6.
24. Wang H, Wang H. Mitigation of Cerebral Ischemia-Reperfusion Injury in Rat by Phellopterin via Antioxidant and Anti-inflammatory Mechanisms. *Iran J Pharm Res.* 2025;24(1):e163643. doi: 10.5812/ijpr-163643.
25. Zhang R, Lei J, Chen L, et al. γ-Glutamylcysteine Exerts Neuroprotection Effects against Cerebral Ischemia/Reperfusion Injury through Inhibiting Lipid Peroxidation and Ferroptosis. *Antioxidants.* 2022;11(9). doi: 10.3390/antiox11091653.
26. Wang Z, Li Y, Ye Y, et al. NLRP3 inflammasome deficiency attenuates cerebral ischemia-reperfusion injury by inhibiting ferroptosis. *Brain Res Bulletin.* 2023;193(March 2022):37–46. doi: 10.1016/j.brainresbull.2022.11.016.
27. Gao S, Qi L, Xu Z, et al. Fujian tablet regulates the Foxo3a/GPX4 axis to promote remyelination and improve motor function in ischemic stroke. *J Ethnopharmacol.* 2026;354(August 2025):120543. doi: 10.1016/j.jep.2025.120543.
28. Alim I, Caulfield JT, Chen Y, et al. Selenium Drives a Transcriptional Adaptive Program to Block Ferroptosis and Treat Stroke. *Cell.* 2019;177(5):1262-1279.e25. doi: 10.1016/j.cell.2019.03.032.
29. Yu W, Lu J, Huang X, Zhuang H, An Y, Zhang M. Exendin-4 promotes ischemia-reperfusion flap survival by upregulating Gpx4 to inhibit ferroptosis. *Eur J Pharmacol.* 2024, 984: 177029. doi: 10.1016/j.ejphar.2024.177029.
30. Bi F, Huang C, Tong J, et al. Reactive astrocytes secrete lcn2 to promote neuron death. *Proc Natl Acad Sci.* 2013;110(10):4069–74. doi: 10.1073/pnas.1218497110.
31. Wang H, Wang Z, Gao Y, Wang J, Yuan Y. STZ-induced diabetes exacerbates neurons ferroptosis after ischemic stroke by upregulating LCN2 in neutrophils. *Experimental Neurology.* 2024;377(11):4797. doi: 10.1016/j.expneurol.2024.114797.
32. Zhao N, Xu X, Jiang Y, et al. Lipocalin-2 may produce damaging effect after cerebral ischemia by inducing astrocytes classical activation. *J Neuroinflammation.* 2019;16(1):1–15. doi: 10.1186/s12974-019-1556-7.
33. Suk K. Lipocalin-2 as a therapeutic target for brain injury: An astrocentric perspective. *Prog Neurobiol.* 2016;144:158–72. doi: 10.1016/j.pneurobio.2016.08.001.
34. Li Y, Lu B, Sheng L, et al. Hexokinase 2-dependent hyperglycolysis driving microglial activation contributes to ischemic brain injury. *J Neurochem.* 2018;144(2):186–200. doi: 10.1111/jnc.14267.